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HEALTH EFFECTS RESEARCH ON MUNITION CONTAMINATED
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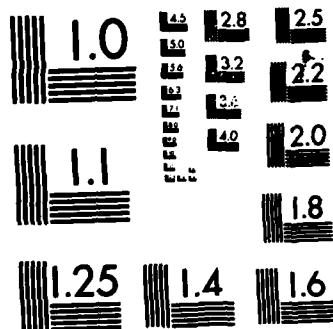
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HEALTH EFFECTS RESEARCH ON MUNITION CONTAMINATED DIMETHYL SULFOXIDE
RECRYSTALLIZATION PROCESS SOLVENT:
PHASE I STUDIES, FINAL SUMMARY REPORT

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JULY 1985

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) <p>Sample material was taken from a dimethylsulfoxide (DMSO) recrystallization process pilot plant and examined chemically and toxicologically, in order to support development of occupational health protection criteria. The report describes work performed by the U.S. Army Medical Bioengineering Research and Development Laboratory and work performed by contractors. The work performed to meet Phase I of the research program has been completed. This includes a problem definition study, the chemical and physical characterization of</p>		

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Octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX)

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)

20. Abstract (continued)

process stream samples and toxicological studies (oral LD50 in rats and mice, primary ocular and dermal irritation in rabbits, acute dermal toxicity in rabbits, dermal sensitization in guinea pigs, Ames assay, and mouse lymphoma mammalian cell assay) on the recycle solvent and evaporator sludge. Since both the latter were identified as having direct-acting mutagens, research objectives for subsequent phases were changed in order to identify the mutagenic agent in the mixtures. *Originator supplied for 10/15*

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PURPOSE AND OBJECTIVES

A comprehensive, three-phase, health effects research workplan was formulated and implemented to develop the essential biomedical data base to support occupational health protection criteria for a new explosives recrystallization process involving potential exposures to dimethyl sulfoxide (DMSO) process solvent mixtures containing dissolved or suspended nitramines. The purpose of this report is to present a summary and assessment of the Phase 1 research plan findings, and to recommend specific changes or clarification in the Phase 2 and subsequent research plan phases based upon the Phase 1 findings.

Specific technical report objectives, therefore, are as follows:

1. Summarize and assess the Phase 1 health effects research plan findings on munition-contaminated DMSO recrystallization process solvent.
2. Review the overall phased or sequenced health effects research plan on the DMSO recrystallization process issue and make changes based on Phase 1 findings.

PROBLEM SETTING BACKGROUND: DMSO RECRYSTALLIZATION OF EXPLOSIVES

The US Army Armament Research and Development Center (ARDC) Large Caliber Weapons Systems Laboratory (LCWSL), is currently conducting a process development evaluation of the replacement of cyclohexanone and acetone with DMSO as the recrystallization solvent employed in a proposed new process design for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) explosives manufacture. This new DMSO recrystallization process concept would result in a continuous or semi-continuous operation as compared to the current batch-type process using cyclohexanone or acetone.

As part of the US Army Manufacturing Methods and Technology Program, process development work was completed over 10 years ago at the laboratory level.¹ This early work revealed that significant increases in recrystallization rates, with corresponding decreases in costs, could be achieved using DMSO. The improvement was attributed primarily to the higher solvent power of DMSO for RDX and HMX as compared with standard acetone and cyclohexanone recrystallization solvents, thereby facilitating larger batch sizes. This early laboratory work led to a second phase pilot plant study using DMSO as the recrystallization process solvent. A DMSO recrystallization pilot plant was designed, and then constructed at Holston Army Ammunition Plant (HSAAP), Kingsport, TN.² The pilot plant was operated for approximately 4 months in late 1979, and included process research on DMSO recrystallization of both HMX and RDX explosives.

The principal process stages and DMSO solvent flow are shown in Figure 1 for the pilot plant operation. The process entailed introduction of both "make-up" industrial grade DMSO (99.8 percent pure from manufacturer) and input of recycled DMSO from a solvent recovery stage of the process. (See General Process Description, Appendix A.) The pilot phase process development

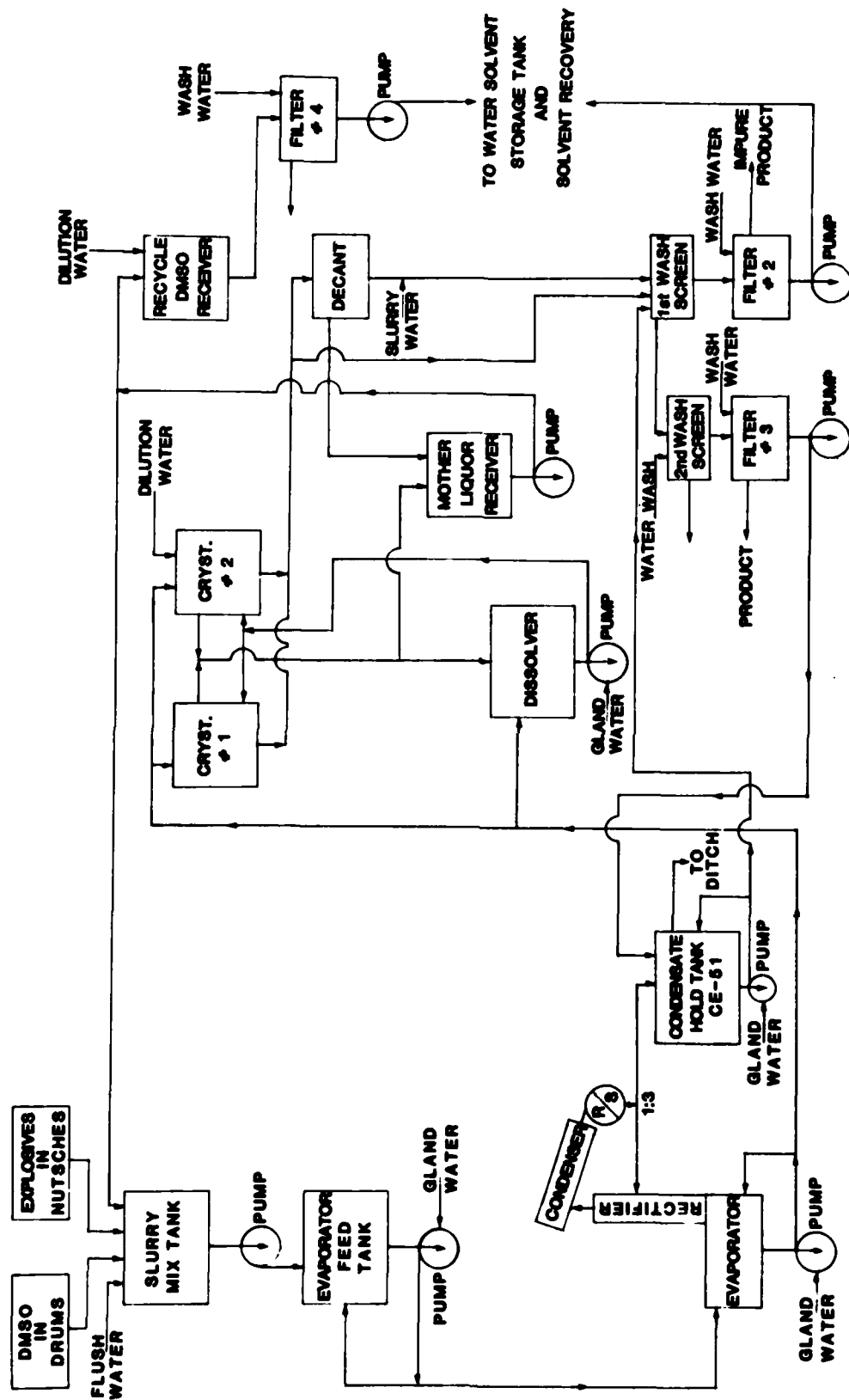


Figure 1. Pilot Plant Process Flow. See Appendix B for Narrative Description (adapted from reference 2).

research produced a data base on engineering design and operating characteristics useful in both further pilot level research and in the full-scale facility design for selected RDX and HMX explosives. Future process development research and facility implementation decisions are dependent upon a number of factors, including: (1) explosives demand, (2) comparative production input costs, (3) qualification testing requirements on new products, and (4) occupational exposure hazards to personnel through routine and accidental exposures to process chemicals.

Critical to the full-scale design and operation of a DMSO recrystallization process for explosives manufacture is a health hazard assessment of potential contaminant exposures and associated control standards to protect workers' health. Particular concern is focused on DMSO as a carrying agent for process stream components through exposed workers' skin due to the rapid absorption of DMSO through body tissue. Process stream worker exposures may occur at several points, ranging from the initial DMSO introduction to process stream sampling, and removal and/or recirculation. The DMSO may be contaminated with varying concentrations of nitramine explosives and related nitramine impurities [RDX, HMX, and smaller amounts of other cyclic nitramines such as octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) and hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine (TAX)], and with other nonmunition-related chemicals in the form of impurities, particularly in the recycle and waste solvent streams.

The Large Caliber Weapons Systems Laboratory, ARDC (formerly the US Army Armament Research and Development Command) recognized at an early stage in their DMSO recrystallization process development research that a thorough health hazard assessment of workplace exposures was necessary. In 1973, a study of the toxicology of RDX and HMX in acetone, cyclohexanone, and pure and technical (or production) grade DMSO was conducted by the US Army Chemical Systems Laboratory (now US Army Chemical Research and Development Center).³ This toxicologic testing, reviewed later in the present report, was conducted with "artificially" created DMSO munition process mixtures by combining production grade DMSO, and pure HMX, and RDX components, as no DMSO recrystallization pilot plant facility had been constructed at that time.

Upon completion of the DMSO recrystallization pilot plant design and operation, and prior to full-scale facility design, the LCWSL, ARDC requested toxicologic testing and health hazard assessment support of the Army Medical Department.⁴ The request focused on development of the necessary biomedical data base for evaluation of potential human exposure to DMSO munition process solvent, to recommend health protection criteria governing process design, and to make operational decisions protective of workers' health. A phased medical research plan was drafted by personnel of the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) to address these occupational health protection issues.

Three types of sample materials were evaluated by the different research projects discussed in this report. All of the materials were collected during operation of the DMSO recrystallization pilot plant in 1979. Assays were performed on this process material at varying times after sample collection, with the last assay performed in 1983. In general, research contractors were requested to keep the sample materials away from sunlight or artificial light,

although there is no guarantee that these provisions were consistently followed. Sample materials consisted of the following:

1. Recycle Solvent. This test material consists of six equal volume collections from two process lines which were combined. Two samples were taken when HMX was being processed and four samples were taken when RDX was being processed (processing of RDX and HMX utilizes the same production facilities). All samples were taken from the recycle DMSO storage tank (see Figure 1) at 35° to 40°C, settled, and allowed to reach ambient temperature and the liquid was decanted for the final sample. Personnel at RAAP have assumed that if the full-scale operation of a DMSO recrystallization plant were automated, the principal target of exposure would be an individual collecting samples for quality control purposes.

2. Evaporator Sludge. The bottom phase of material in the evaporator (Figure 1) was drawn after the pilot plant operation was completed.

HEALTH EFFECTS RESEARCH PLAN AND APPROACH

The research plan was developed to establish a biomedical data base for occupational health criteria. The plan recommended by USAMBRDL and supported by LCWSL consisted of three phases:

Phase 1. The initial phase establishes the minimum information necessary to characterize the health hazard of the DMSO-nitramine mixture. Typically a minimal data base is generated by reviewing the literature, performing chemical/physical analysis, performing acute toxicity assays and performing screening assays to evaluate mutagenic potential. This phase may establish that sufficient information is available to support criteria recommendations or may determine that additional information is needed.

Phase 2. Additional evaluations are usually required when the literature lacks adequate documentation of tests for long-term effects (chronic studies), especially for effects resulting from the anticipated routes of exposure (i.e., in the present case through the skin) and when there have been positive findings from the acute toxicity or mutagenic screening assays. Evaluations may include subchronic studies, isolation of the biologically active fraction of the mixture, and studies to confirm mutagenicity and/or establish carcinogenic potential.

Phase 3. The last phase attempts to further evaluate the potential for long-term chronic toxicity and carcinogenicity to humans. Because these studies are costly and time consuming, it is often necessary to make a cost/benefit decision that balances the need for continued production and the associated exposure against the expected risk of adverse health consequences.

Phase 1 research consisted of the following:

a. Problem Definition Study. A comprehensive literature review of available scientific and biomedical data on DMSO and the major recrystallization process stream contaminants was performed by Oak Ridge National Laboratory's Chemical Information Center.⁵ Principal emphasis was placed on the interaction of DMSO and dissolved substances and the resultant health effects.

b. Chemical and Physical Characterization of Process Stream Samples. Chemical and physical analysis of the process stream samples was used initially to estimate the concentration of chemical substances that a worker might be exposed to. These preliminary investigations also served to identify unexpected impurities and establish the range of dose to be used in toxicological assays. Holston Defense Corporation provided samples of evaporator sludge and recycle solvent to USAMBRDL for analysis.

c. Toxicology. In general, the most efficient approach is to conduct toxicological evaluations on mixtures that represent potential exposures. If the toxicological tests define no significant deleterious effect on test animals, then a compound-by-compound evaluation of the mixture components is usually not necessary. Additionally, testing of mixtures identifies synergistic or antagonistic effects not predictable from single-component evaluations alone.

(1) Two laboratories performed toxicological evaluations. The evaluations consisted of acute toxicity and mutagenicity screening, generally involving a single dose or exposure administered over a short time. (Such studies are usually conducted as a starting point in the health hazard assessment process.) They addressed several key objectives:

(a) To give a quantitative measure of acute toxicity (LD50) for comparison with other substances,

(b) To identify the clinical manifestations of acute toxicity,

(c) To provide dose-range guidance for other tests.

(d) To screen for potential mutagenicity.

(2) The Letterman Army Institute of Research performed the following tests on pure DMSO, recycle solvent, and evaporator sludge:

(a) Ames Assay (plate incorporation triplicate) - a mutagenic potential assay

(b) Oral LD50 in rats and mice

(c) Primary ocular and dermal irritation in rabbits

(d) Acute dermal toxicity in rabbits

(e) Dermal sensitization in guinea pigs

(3) The Laboratory for Energy-related Health Research, University of California, Davis, performed an in vitro mouse lymphoma mammalian cell assay. The mammalian cell (eukaryotic) assay is a better risk estimator than that derived solely from a prokaryotic cell (the Ames test utilizes a prokaryotic cell).

PHASE I RESEARCH FINDINGS AND DISCUSSION

DMSO PROBLEM DEFINITION STUDY

Typically, prior to conducting any research, an important first step is a literature review.⁶ The objectives of the review were to identify and define the known mechanisms of action and biological effects of DMSO when combined with other chemicals; to define the similarities of these other chemicals to nitramines of military concern and to briefly characterize the range of biological effects of DMSO.

DMSO's properties are such that a wide range of interactive effects has been described. Administration of DMSO with another substance orally, intravenously, or intraperitoneally either increases, decreases, or has the same response as the substance's activity alone. There are even circumstances where the included substance modifies the effects of DMSO itself.

DMSO's ability to act as a carrier for a wide range of substances through the skin has also been widely observed, but there did not appear to be any information facilitating prediction of effects through knowledge of DMSO's interaction with a particular class of chemical compounds.

Only one study involving explosives (RDX and HMX) administered in DMSO was available in the literature. McNamara et al.,³ established the toxicity of RDX and HMX in DMSO, but unfortunately comparable doses could not be administered at the same concentration in cyclohexanone and acetone to determine comparative effects. Despite these shortcomings, data were obtained on the physiological response to RDX and HMX in DMSO administered both intravenously and cutaneously. Skin administration did not elicit a significant response, but both cardiovascular and central nervous system effects occurred from DMSO with RDX and HMX introduced intravenously in laboratory animals.

In summary, the literature review did not identify any physical or chemical characteristics common to those substances enhanced, diminished, or unchanged by DMSO. Observed interactions, in general, were dependent upon the chemicals and their state (liquid or solid). Most of the interactive effects reported were as a result of the enhancement of adverse effect(s) via dermal administration. In such cases the most important variable was the concentration of the test substance in DMSO. The review did not lead to any rules for predicting how a particular substance's effects would be modified by the presence of DMSO. Considering the many cases of increased response, one should assume that an untested substance would penetrate the skin faster in the presence of DMSO.

CHEMICAL/PHYSICAL CHARACTERIZATION OF DMSO PROCESS SOLVENT

Evaporator sludge and recycle solvent were analyzed for trace organics following findings positive for mutagenicity.^{6,7} The chemical analysis and procedures are detailed in USAMBRDL Technical Report 8407.⁸ Results are summarized in Table 1. The report identified diacetone alcohol as a product of aldol condensation of acetone; dimethylsulfone was an obvious oxidation product of DMSO. Benzothiazole is a commercially available chemical, and the presence of DDBH has been reported in highly polluted rivers in Japan. No impurities were found in the DMSO before use.

TABLE 1. HPLC ANALYSIS OF MUNITIONS FROM DMSO RECRYSTALLIZATION
PROCESS SAMPLES, JUNE 1983

Compound	Retention Time (min)	Recycle Solvent Amt (ppm) or Presence	Evaporator Sludge Amt (ppm) or Presence
<u>Nitramines</u>			
SEX	7.3	150	a
HMX	9.1	3,500	a
TAX	11.2	500	a
RDX	15.7	5,500	a
<u>Trace Organics</u>			
Diacetone alcohol	2.4	+	- ^b
Dimethyl sulfone	4.1	-	+
Benzothiazole	4.9	+	+
1,5-Di-t-butyl-3,3- dimethylbicyclo (3.1.0) hexan-2-one (DDBH)	6.8	+	+

a. Relative amounts in June 1983 sample were TAX > RDX > HMX >> SEX

b. Not detected because of DMSO interference, but believed to be present.

A literature review of the trace organics indicated that diacetone alcohol has a potential for genetic effects⁹ and that benzothiazole has previously demonstrated mutagenic effects.¹⁰

The chromatograms of the evaporator sludge, the recycle solvent, and the analytical procedures for analyzing the samples are provided in Appendix B. These chromatograms allow future comparisons with additional pilot plant samples or full-scale process samples.

ACUTE TOXICITY ASSAYS

A series of studies was carried out on the DMSO process stream samples, i.e. the evaporator sludge, the DMSO recycle solvent and virgin DMSO itself. Acute oral toxicity in rats:¹¹ The evaporator sludge and DMSO were nontoxic up to the limit dose-level of 5.0 mL/kg bwt while the recycle solvent was more toxic, with an LD50 for male rats at 2.1 mL/kg bwt and for female rats at 1.3 mL/kg bwt (for details see Appendix C, reference 3). Similar studies with mice¹² produced like results although the virgin DMSO produced some mortalities (15% in males; 29% in females). The recycle solvent had an LD50 of 4.0 mL/kg bwt in male mice and 2.5 mL/kg bwt in female mice (see details in Appendix C, reference 2).

The acute toxicity would not appear to be due to the presence of SEX, which has been shown to have an LD50 greater than the limit dose of 5.0 mL/kg bwt¹³ in rats (see Appendix C, reference 4 for details). There are no acute toxicity data available for TAX. Similarly, the presence of HMX, which has an

LD50 in rats of 7.3 g/kg bwt and 2.7 g/kg bwt in mice (unpublished data), probably would not contribute to the acute toxicity. The acute oral LD50 value for RDX in rats is 118.1 ± 2.8 mg/kg, and in mice is 80.3 ± 9.6 mg/kg bwt.¹⁴ Since these values are more in agreement with the values found for the sludge and the recycle solvent in particular, it is probable that this toxicity is due to the presence of RDX only.

The acute dermal toxicity potential of the evaporator sludge, the recycle solvent, and DMSO has also been studied.¹⁵ Topical application of the three solutions to the skin of New Zealand white rabbits produced only minimal irritation, with no animal deaths at the limit dose of 2 mL/kg bwt (see Appendix C, reference 5 for details). A similar study for primary eye irritation in rabbits was also carried out.¹⁶ Again, some irritation was noted, but the three solutions could not be classified as positive eye irritants (See Appendix C, reference 6 for details).

The potential for dermal irritation of the three solutions was studied in New Zealand white rabbits.¹⁷ Some slight erythema on a few animals was noted but no irritation was observed after one application of the test solutions (see Appendix C, reference 7 for details). A final study was carried out to assess the potential for dermal sensitization of the three solutions.¹⁸ The solutions showed some mild irritancy, but provided little evidence of a sensitizing potential under the standard test conditions in guinea pigs (see Appendix C, reference 8 for details).

The dermal sensitization of SEX was also evaluated in the standard guinea pig sensitization test.¹⁹ SEX was found to be a nonsensitizer under the test conditions (see Appendix C, reference 9 for details).

MUTAGENICITY ASSAYS

The mutagenic potential of the Holston compounds (evaporator sludge, recycle solvent, and DMSO) has been tested in two assay systems, i.e. the Ames Salmonella/microsome assay⁶ and the mammalian mouse lymphoma assay.⁷

In the Ames Salmonella assay,⁶ all five tester strains gave a negative test for the virgin DMSO and the recycle solvent, but a positive response was obtained for the DMSO evaporator sludge (see Appendix C, reference 10 for details). In the mouse lymphoma assay,⁷ both the evaporator sludge and the recycle solvent showed positive mutagenic activity, with the recycle solvent being relatively high in mutagenic potency when compared with known carcinogens. The virgin DMSO had insignificant mutagenic activity above 4% volume/volume and was cytotoxic at 10 percent volume/volume. The mutagenic activity was not affected by the presence of the S-9 liver activation factor (see Appendix C, reference 13 for details).

In an attempt to relate the identified mutagenic activity to compounds known to be present in the solutions (see Table 1), the Ames Salmonella assay was used to test both SEX and TAX.²⁰ Both pure compounds proved to be negative in all five tester strains (see Appendix C, reference 11 for details). Previous studies on the mutagenicity evaluations of RDX and HMX according to the Ames Salmonella microsome activation assay have been reported. Cholakakis et al.¹⁴ and Epler²¹ reported that RDX was negative when tested with the five standard strains of *S. typhimurium*, both with and without S-9 rat liver activation. Similar tests with HMX reported by Epler²¹ and by

Whong et al.²² were also negative. The compound benzothiazole was similarly tested.²³ It proved to be negative in all five strains of Salmonella at doses ranging from 1 μ L/plate to 3×10^{-4} μ L/plate (see Appendix C, reference 12 for details). The negative Ames assay for pure (100%) benzothiazole is at variance with a recent Japanese report.¹⁰ The results reported here are for all five Salmonella tester strains whereas the Japanese report used only one strain of S. typhimurium i.e., TA 100. The purity of the benzothiazole used was not given.

PHASE 1 RESEARCH CONCLUSIONS

The literature review characterized DMSO as a solvent that is able to carry other dissolved substances easily into the body through the skin. Other interactive health effects are not well understood. With the exception of RDX, most of the contaminant chemicals dissolved in DMSO are inadequately documented as to human health effects. Chemical analysis efforts identified the expected DMSO, H₂O, RDX, HMX, SEX, and TAX as well as four unexpected compounds: dimethyl sulfone, benzothiazole, diacetone alcohol, and DDBH.

Acute toxicity results for virgin DMSO did not demonstrate significant results. Erythema in a dermal assay (rabbit), irritation for the eye assay (rabbit), and for the skin assay (guinea pig) can be characterized as minor. Acute oral LD50 in rats proved the evaporator sludge to be slightly toxic and the recycle solvent moderately toxic. The mutagenicity screening tests suggest that both the recycle solvent and the evaporator sludge samples be considered direct-acting mutagens.

PHASE 2 RESEARCH PLAN RECOMMENDATIONS

Decisions concerning future work are complicated by three main issues:

- a. What procedures are necessary to determine the source of observed mutagenic activity?
- b. What toxicological evaluations should be conducted to support setting of occupational health standards until the source of mutagenic activity is accounted for?
- c. What consideration should be given to evaluations of pilot plant sample results determined during Phase 1 as compared to evaluations during Phase 2 (i.e., What significance can be placed on sample "aging" in storage drums at Holston?) What steps should be taken to ensure that research performed on pilot plant samples can be compared to future pilot plant samples or samples from production facilities.

Issue a. appears to be predominate. If the DMSO recrystallization process contains mutagenic components that are inherent to the process, the process potentially could be rejected because of the hazard alone or because the cost of providing engineering controls would prove to be detrimental in a cost/benefit analysis. Toxicological evaluations to determine a worker protection

standard would then be of secondary importance. Careful design of the mutagenic/carcinogenic assays might permit information incidental to those assays to be used in support of standards setting.

Design of studies to take into account "aging" of the original pilot plant samples may not be possible, at least for comparing toxicological results. Emphasis on chemical characterization of all samples will provide the best basis for comparison among samples.

OBJECTIVES FOR PHASE 2 MUTAGENICITY ASSAYS OF PROCESS SOLVENT COMPONENTS

Mutagenic/carcinogenic assay strategies for complex mixtures can be compartmentalized into three areas of approach:

- a. Evaluate the mutagenic/carcinogenic potential of the known, individual components of the mixtures.
- b. Create artificial mixtures of the known components and compare the results to the original mixture.
- c. Fractionate the original mixture, sequentially identifying the active fractions, until the active component can be identified by chemical analysis.

Optimally the research can take each of these approaches into an integrated approach (mutagenic potential of RDX, HMX, SEX, TAX, dimethyl sulfone, diacetone alcohol, and benzothiazole).

The objectives for mutagenicity/carcinogenicity assays for Phase 2 are as follows:

- a. To determine the mutagenic/carcinogenic potential of known components in the DMSO process solvent that have not been previously tested in this regard (diacetone alcohol, and dimethyl sulfone).
- b. To determine the mutagenic/carcinogenic potential of an artificial mixture using pure components in order to identify possible interactions between the components. Mixture components would include DMSO, water, RDX, HMX, SEX, TAX, diacetone alcohol, benzothiazole, and dimethyl sulfone.
- c. To determine which component of the recycle solvent and the evaporator sludge contains identifiable mutagenic activity. Fractions of the solvent and the sludge would be selected by gas chromatography/mass spectroscopy technique and individually subjected to mutagenic assays.

OBJECTIVES FOR PHASE 2 CHEMICAL CHARACTERIZATION OF PROCESS SOLVENT

Only dimethyl sulfone clearly originated from reaction of the solvent, DMSO. None of the other trace organics identified can be attributed either to the munitions manufacturing process or to breakdown or transformation of the munitions compounds. Diacetone alcohol could have been a solvent-derived product in the acetone/cyclohexanone recrystallization process, but not in the DMSO process. Evaluation of the treated Holston River water and the cyclohexanone/acetone appears appropriate.

Objectives for Phase 2 chemical characterization include:

a. Screen samples from the cyclohexanone/acetone process in order to determine if the trace organic impurities found in recycle solvent and evaporator sludge are present. It has been suggested that the DMSO samples may have been contaminated after the explosives were nutsche (suction-filtered) in G-building recrystallization equipment.

b. Evaluate the contribution of trace organics from processed Holston River water. Water samples must be obtained from an intake stream that comes in contact with the recrystallization solvent. River water impurities, however, change continually, responding especially to changes due to upstream discharges.

c. Provide analytical profiles suitable for future comparison to new pilot plant samples and production plant samples.

OBJECTIVES FOR PHASE 3 TOXICOLOGY OF PROCESS SOLVENT

The original objectives for Phase 2 research (see the text on page 14) called for evaluation of the toxicity of individual components in each of the mixtures, teratogenicity, subchronic toxicity, and toxicokinetics. This approach has been abandoned for Phase 2 in order to resolve the positive mutagenicity findings. If mutagenicity is confirmed in the DMSO process, three options are available:

a. Eliminate the source of mutagenic activity if practically and fiscally feasible. Selection of this option may require additional chemical and toxicological assays to determine if process changes were successful. These evaluations would be followed by assays to establish criteria based on acute and chronic considerations. This option would return the research to the original objectives envisaged under Phases 2 and 3 at the beginning of the project.

b. Provide engineering controls to delete or minimize exposure of workers to potentially carcinogenic material. These engineering controls would conform to OSHA guidelines for exposure to carcinogenic materials. Chemical and toxicological evaluations would be required to demonstrate that the exposure potential had been reduced or eliminated.

c. Terminate biological testing.

If mutagenicity is not confirmed, all previous results become ambiguous. No further purpose would be achieved by continuing assays on the original pilot plant material. Additional medical research would only prove useful if new pilot plant samples were produced, in which known sources of contamination had been eliminated.

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APPENDIX A

DIMETHYL SULFOXIDE (DMSO) RECRYSTALLIZATION PILOT PLANT

GENERAL PROCESS DESCRIPTION* (Reference Figure 1)

Feed was prepared in the slurry mix tank by mixing crude explosives, recycle solutions of varying composition, and make-up DMSO. The mixture was pumped to the evaporator feed tank and then metered into the evaporator/rectifier system. DMSO was concentrated in the evaporator by removing water as an overhead stream from the top of the rectifying column. Concentrated DMSO/explosives solution in the evaporator became the feed for the batch, controlled nucleation, classified product removal (CNCPR) crystallization system. The ratio of explosives to DMSO as well as the evaporator conditions were controlled so that the feed solution was maintained at 85 to 90 percent DMSO by weight and contained no undissolved explosives. This feed material could be introduced to the CNCPR system at several points, depending on the particular product to be produced. Generally it was metered directly into the crystallizers or the dissolver. Conditions within the CNCPR system were controlled to cause precipitation of explosive crystals. This was accomplished by water quenching to produce fine particle size distributions (PSD's) or by simply cooling the feed solution to produce the coarser PSD's. The conditions chosen determined both the particle size distribution and throughput rates obtainable.

Coarse explosives (Classes 1, 3, and 4) produced by cooling techniques were removed from the crystallizers at timed intervals in a thickened slurry containing approximately 40 percent solids and 60 percent spent solvent. The liquid was decanted into the mother liquor receiver and held at ambient temperature until transferred for recycle solvent processing or reused directly for making new feed solution. After decantation of the liquid, the solids were diluted with water to reduce the solvent concentration and to precipitate the remaining dissolved material. Reslurried product was then fed to the first wash screener and spray washed with a dilute (0.5 to 2 percent) solution of DMSO in water. The underflow fines obtained from the screening operation were collected, filtered, and washed with clean water in a vacuum filter. Filtrate was transferred to the waste solvent storage tank. The bulk of the product crystals overflowed onto the second wash screener and were washed again with clear water. The combined overflow and underflow were collected in another vacuum filter, dewatered and then placed in product containers. The filtrate was combined with the fractionating column distillate in the condensate receiver and used for first wash water. The excess was discarded.

Recycle DMSO collected in the mother liquor receiver was used directly to make new feed solution or processed as recycle solvent. Material transferred to the recycle DMSO receiver was cooled to 20° to 25°C (293 to 298 K) to precipitate the remaining explosives. The solids were allowed to settle out in the receiver and the liquid overflowed to the recycle DMSO holding tank. The settled solids in the receiver eventually required filtration in filter

* Adapted from Rothrock, et al. 1981 (reference 2).

#4. The filtrate was combined with the receiver overflow in the holding tank, where it was stored until required for making new feed. The solids in filter #4 were water washed and stored as waste explosives. The wash water was transferred to the waste solvent storage tank.

Waste solvent generated from the various product washing operations, plus the solvent purge, generally contained 10 to 30 percent DMSO and 1 to 2 percent explosives by weight. Recovery of the DMSO involved filtering the slurry in the waste solvent filter to remove explosives and other solid impurities, fractionating the resulting solution to remove water and other volatile impurities and to concentrate the DMSO, and evaporating the concentrated DMSO as an overhead distillate to free the DMSO of dissolved impurities. The evaporator temperature, the column pressure, and the reflux ratio were controlled to obtain the desired concentration of DMSO in the evaporator and distillate. In general, the DMSO was concentrated to 95 to 98 percent, while the distillate contained less than 0.01 percent DMSO. The concentrated DMSO was collected in storage tanks and later fed to the evaporator, where it was evaporated as an overhead product and collected as 85 to 86 percent DMSO. (Note: The remaining 14 percent of this distillate is water that enters the system through the evaporator circulation pump packing gland.)

APPENDIX B

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF DMSO SLUDGE AND SOLVENT SAMPLES

1. DMSO EVAPORATOR SLUDGE SAMPLE.

Sample Preparation. Two 1-mL portions of the DMSO sludge sample were pipetted into volumetric flasks and made up to 100 mL and 500 mL with glass-distilled/deionized water. Four mL of each diluted sample was passed through a 0.45 μ m MILLEX-SR FILTER into a WISP sample vial and assayed by direct-injection reversed-phase high performance liquid chromatography.

HPLC conditions (DMSO sludge sample)

Column: ZORBAX C8

UV-254 nm, 0.05 AUFS

Injection Volume: 200 μ L

2. DMSO RECYCLE SOLVENT SAMPLE

Sample Preparation. One milliliter of the DMSO solvent sample was made up to 100 mL with uv grade acetonitrile, and 10 mL of this solution was made up to 100 mL with uv grade acetonitrile. Four milliliters of each diluted sample was passed through a 0.45 m MILLER-SR FILTER FILTER into a WISP sample vial and assayed by direct-injection reversed-phase high performance liquid chromatography.

HPLC Conditions (DMSO solvent sample)

Column: ZORBAX C8

Mobile Phase: Pump A-20% Methanol/Water

Pump B-80% Methanol/Water

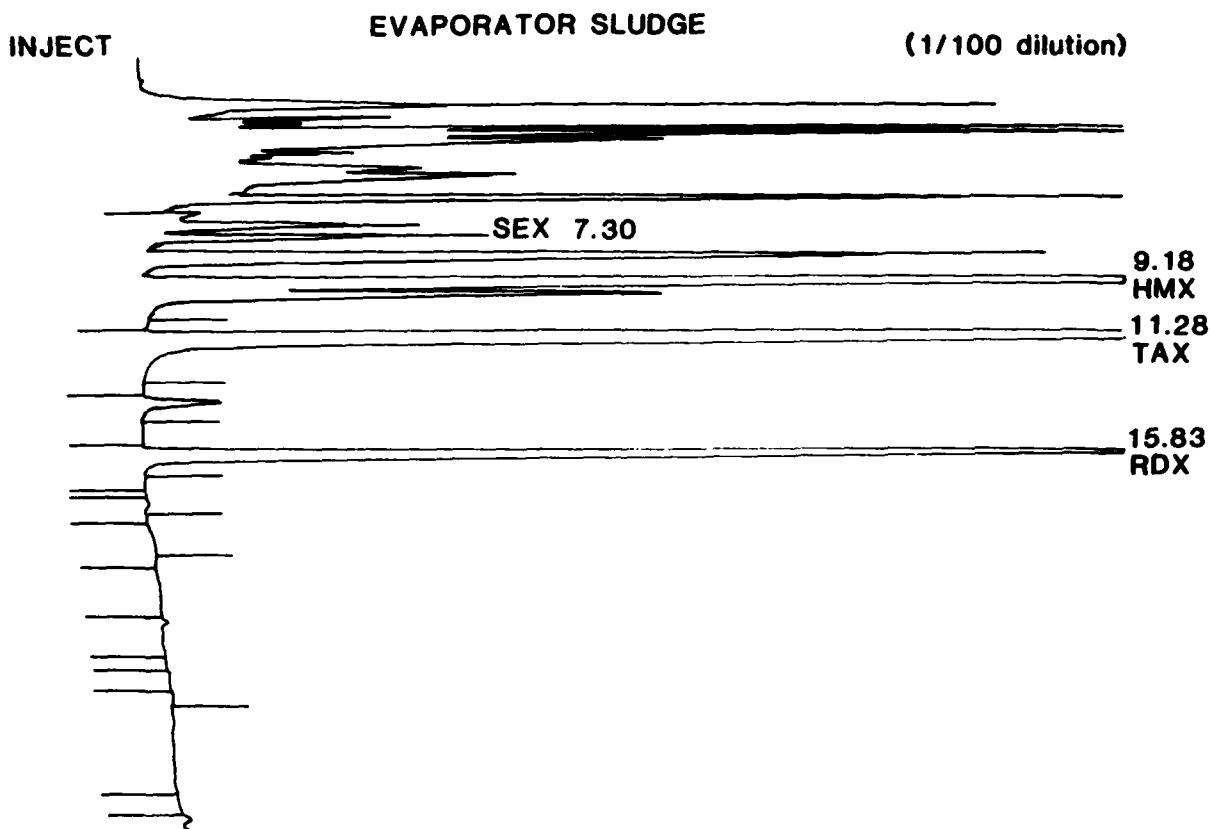
Gradient: 5% B-50% in 25 min

Flow Rate: 2.1 mL/min

UV-254 nm, 0.05 AUFS

Injection Volume: 25 μ L

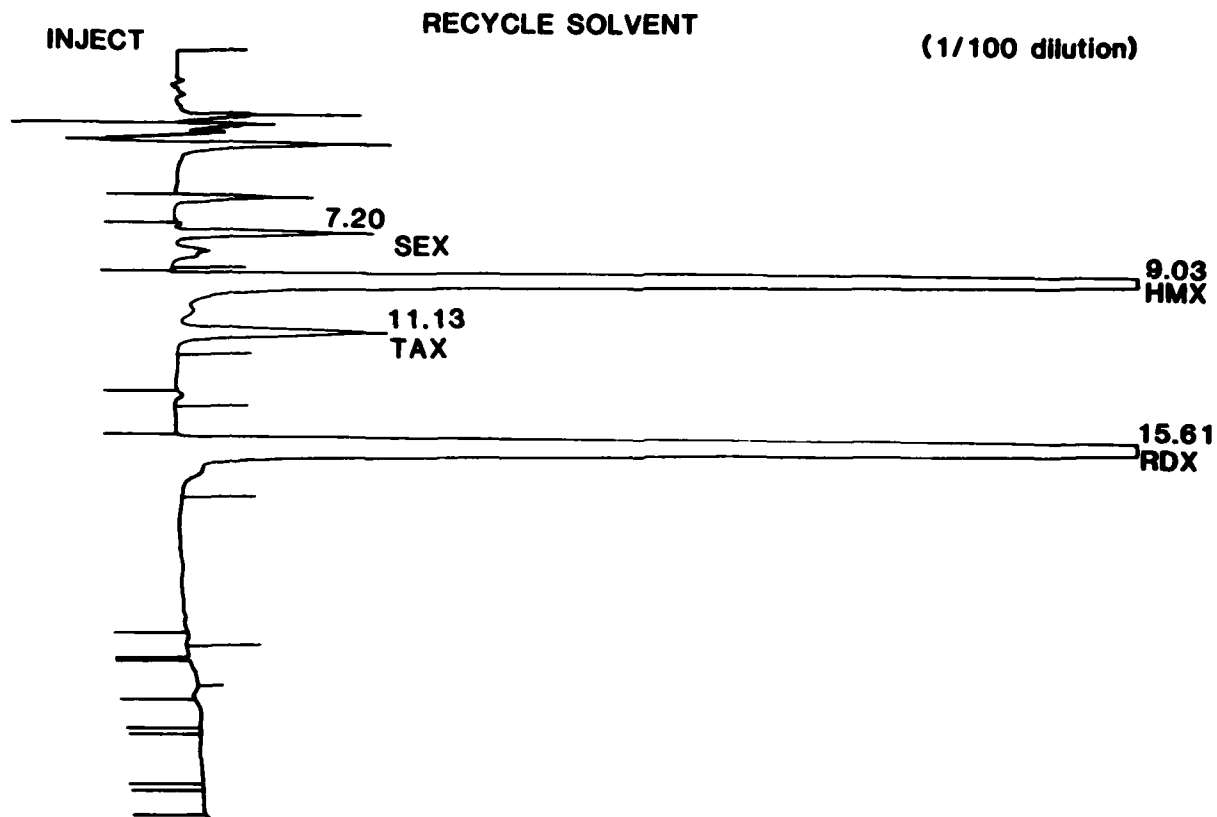
3. Chromatograms and calculated concentrations are contained in Figures B-1, B-2, and B-3.



ASSAY DATE: 1/23/84

SAMPLE	RESULTS			
	PPM SEX	PPM HMX	PPM TAX	PPM RDX
DMSO EVAPORATOR SLUDGE	64	1,751	2,402	403

Figure B-1. Evaporator Sludge.



ASSAY DATE: 1/24/84

SAMPLE	RESULTS			
	PPM SEX	PPM HMX	PPM TAX	PPM RDX
DMSO RECYCLE SOLVENT	412	42,883	648	39,851

Figure B-2. Recycle Solvent.

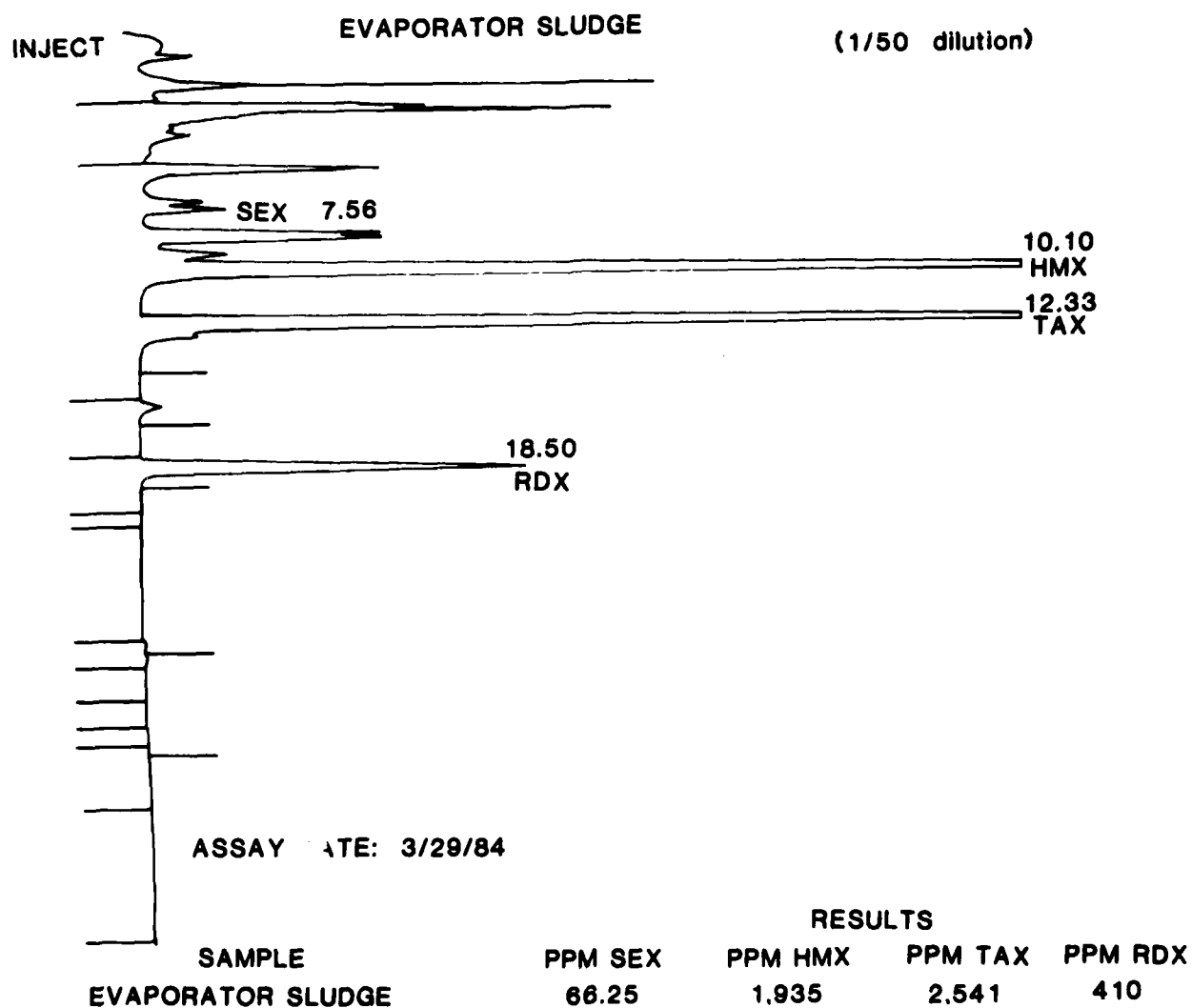


Figure B-3. Evaporator Sludge.

APPENDIX C

ABSTRACTS AND SUMMARIES FROM CONTRACTOR REPORTS

Abstracts and summaries from contractor reports of work on DMSO and the Holston Compounds that have been reviewed for this document.

1. Smith, L.H., D.M. Oprosko, J.W. Holleman, and R.H. Ross. 1983. Problem Definition Study of Dimethyl Sulfoxide (DMSO) and Interactive Health Effects with Other Chemicals. Final Report, ORNL-EIS-203, AD 140554. Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Dimethyl sulfoxide (DMSO) is a versatile molecule, with interesting physical and chemical properties. It is quite stable under normal conditions, but can be oxidized, reduced, and decomposed by vigorous means. Physically, the high degree of self-association of DMSO causes it to have a high boiling point (189°C) and to be a liquid over a wide temperature range. The ability of DMSO to be polarized without dissociation is the basis for its ability to act as a solvent for a wide range of chemicals. As a chemical reactant, DMSO can act either as an electrophile (at the positive S atom) or as a nucleophile (at the negative O atom); most often it acts as the latter. Various chemical reactions are possible, including substitutions, dismutations, and cyclo-additions. DMSO makes dipole-dipole complexes with a number of chemical entities (e.g., nitriles and ketones), charge transfer complexes with others (e.g., iodine and iodine-halogen compounds), and coordination compounds with nearly all inorganic cations. Particularly noteworthy is the association of DMSO with transition states of reactants and its solvation of reactive species. As a "super solvent" DMSO promotes the rate of bimolecular reactions, allowing these to achieve their true rates, without the hindrance observed in other solvents. The electron-donating function of DMSO allows it to participate in H-bonding as an H-bond acceptor. This, as well as its effect on the structure of water, and its complexing ability, is the basis for its effects on biological molecules and in biological systems.

The chemical properties of DMSO allow it to pass rapidly through biological membranes, and it can easily be taken up through the skin. Accidental spillage of the solvent would not, however, pose a major health hazard, because, of itself, DMSO has a relatively low level of acute toxicity. Clinical studies indicate that cutaneous doses as high as 1 g/kg/day of an 80 percent DMSO gel, even if given for a 3-month-long period, would not produce signs of toxicity. Tolerance limits, however, have not been established for all exposure conditions. Higher doses, more concentrated solutions, a greater frequency of exposure, or a longer exposure period may produce one or more of the toxic effects seen in some laboratory and clinical studies; these include (1) scaling erythematous dermatitis; (2) damage to the epithelial tissue of the lungs following inhalation; (3) teratogenic effects; (4) increased frequency of chromosomal aberrations; (5) lenticular changes in the eye; (6) hemolysis, and (7) biochemical indications of hepatotoxicity and possibly nephrotoxicity, although the latter might be a secondary effect of DMSO-induced hemolysis. At present there is no clinical evidence that DMSO is teratogenic in humans or that it causes ocular changes in humans, but further study is warranted. Substantial data, however, indicate that it is not mutagenic or carcinogenic.

Laboratory studies have also shown that secondary to its toxic effects, DMSO (1) acts as a strong diuretic; (2) alters muscle tone and muscle response to stimulation in vitro and can cause vasodilation; (3) is an anti-acetylcholinesterase agent, but high concentrations may block cholinergic transmissions; (4) has analgesic properties; (5) stimulates adrenal and pituitary gland secretions in vitro, but may not do so in vivo; (6) has little effect on metabolic rate, but may influence lipolysis, protein synthesis, and some enzyme activities; and (7) increases membrane permeability and thus enhances intracellular and extracellular transfer of chemical compounds through the body.

Because of its effects on membrane permeability and its excellent solvent properties, DMSO has been used as a solvent vehicle for a wide variety of substances administered to humans and to several species of laboratory animals. While the broad solvent properties of DMSO have been the chief reason for its extensive application, results of many experiments demonstrate that it can modify the biological response to substances for which it has been employed as a vehicle. The observed modifications reflect rather poorly understood interactions that involve DMSO, the substance, and the target tissue.

DMSO can alter the response to many classes of substances including carcinogens, hepatotoxins, teratogens, mutagens, steroids, allergens, dyes, cytotoxins, and a wide variety of drugs. In many instances, the alteration involves an increase in the magnitude of the response, particularly in cases involving percutaneous absorption of a substance. The oral, intraperitoneal, or intravenous mode of administration of DMSO with a substance, however, often results in an unaltered response; and in some situations a reduction of response can be attributed to DMSO. Aside from their solubility in DMSO, there do not appear to be any physical or chemical characteristics common to those substances whose action is enhanced, diminished, or unchanged by this vehicle. Consequently, it is not possible at present to predict from knowledge of a substance's physical and chemical properties if and to what extent DMSO might modify its action. It would seem judicious to assume that an untested substance would penetrate the skin faster when in the presence of DMSO.

How the response to a particular substance is altered by DMSO is not clear, but generally it is thought to act as a penetrant carrier of substances through membranes at all levels of biological organization. Two important properties of DMSO as a penetrant carrier involve its ability to change conformation of proteins and to replace water; either action can alter penetration rates of substances dissolved in DMSO. Changes in penetration rates of a substance through a membrane constitute a likely basis for an altered response to that substance. Although there is some evidence for a direct chemical reaction between DMSO and its solute, these kinds of reactions do not appear to play a major role in DMSO-induced alteration of the response to most substances tested.

In an assessment of the interactive health effects of DMSO and another liquid, a direct comparison can be made between the liquid alone and the liquid administered in conjunction with DMSO. However, it is not possible to make the same type of comparison for solid substances, for which assessment can be made only relative to the solid in a vehicle other than DMSO.

Therefore, DMSO diminishes, increases, or has no effect on the biologic response to a substance only relative to the response of that substance administered in another vehicle.

A most important experimental variable related to the action of DMSO on the response to a substance is the concentration of DMSO used. In general, high concentrations of DMSO are tantamount to maximum alterations of the response, especially those responses associated with percutaneous absorption.

At present there are no known restrictions to using DMSO as an industrial solvent. Although there is substantial evidence for its therapeutic value in a variety of human disorders, prior considerations of its potential toxicity have prevented its being licensed for use as a drug except in the treatment of interstitial cystitis. However, with increasing data supporting the contention that it is therapeutically effective without significant toxic side effects, it may soon be licensed by the U.S. Food and Drug Administration for use in the treatment of scleroderma and muscle-skeletal disorders.

Finally, it should be noted that little information exists on the effects of subchronic and chronic percutaneous exposure to DMSO in combination with other agents. In view of the conditions that would be present in the workplace, further studies of this kind would seem to be in order for both RDX and HMX. Agents chosen for study should be those to which personnel may be expected to be exposed.

2. White, C.W., J. Rodriguez, and G.E. Marrs, Jr. 1983. Acute Oral Toxicity of DMSO Process Stream Samples in Male and Female Mice. Institute Report No. 167, AD A137228. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The acute toxicities of single oral doses of the DMSO process stream samples, DMSO evaporator sludge, DMSO recycle solvent, and virgin DMSO were determined in male and female albino ICR mice. The DMSO evaporator sludge solution produced no deaths in male or female mice at the highest dose level, 5.0 mL/kg. The virgin DMSO solution produced mortalities in one of seven males (14%) and two of seven females (29%) at the top dose of 5.0 mL/kg. The DMSO recycle solvent LD50 with 95 percent confidence limit was calculated by probit analysis. The DMSO recycle solvent LD50 was 4.0 mL/kg in male mice, 95 percent confidence limit (3.2 mL/kg, 5.1 mL/kg) and 2.5 mL/kg in female mice, 95 percent confidence limit (2.2 mL/kg, 2.8 mL/kg).

3. White, C.W., J. Rodriguez, and G.E. Marrs, Jr. 1983. Acute Oral Toxicity of DMSO Process Stream Samples in Male and Female Rats. Institute Report No. 164, AD A137309. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The acute toxicities of single oral doses of the DMSO process stream samples, DMSO evaporator sludge, DMSO recycle solvent, and virgin DMSO, were determined in male and female albino Sprague-Dawley rats. The DMSO evaporator sludge and virgin DMSO solutions produced no deaths in male or female rats at a limit dose level of 5.0 mL/kg. The DMSO recycle solvent was more toxic, and an LD50 with 95 percent confidence limit was calculated by probit analysis. The DMSO Recycle Solvent LD50 was 2.1 mL/kg in male rats, 95 percent confidence limit (1.5 mL/kg, 2.8 mL/kg), and 1.3 mL/kg in female rats, 95 percent confidence limit (1.0 mL/kg, 1.8 mL/kg).

4. White, C.W. and E.M. Zimmerman. 1984. Acute Oral Toxicity of 1-Acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) in Male and Female Rats. Institute Report No. 171, AD A142351. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The acute oral toxicity potential of the explosives by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), was determined in male and female albino Fisher 334 rats by using a single-dose, free-choice feeding method. The study was conducted in compliance with the Good Laboratory Practice Regulations. No compound-related mortality was observed at a limit dose of 5.0 g/kg.

5. Mullen, L., C.W. White, and G.E. Marrs. 1984. Acute Dermal Toxicity Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge in Male and Female Rabbits. Institute Report No. 174. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The acute dermal toxicity potential of the Holston Compounds (Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge) was determined in rabbits by topical application to skin sites with plastic covering over the exposed areas for 24 hours. There were no compound-related deaths at a limit dose of 2 mL/kg during this study. The Holston Compounds caused minimal dermal irritation.

6. Kellner, T.P., C.W. White, and J.T. Fruin. 1983. Primary Eye Irritation of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge. Institute Report No. 155, AD A132407. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The primary eye irritation potential of the Holston Compounds (virgin DMSO, DMSO recycle solvent, and DMSO evaporator sludge) was tested by placing these materials in the conjunctival cul-de-sac of rabbit eyes. The study was conducted in compliance with the Good Laboratory Practice Regulations. All of the DMSO compounds tested produced irritation of the conjunctiva early in the study, but the level of severity did not meet the criteria for positive eye irritants.

7. Lewis, C.M. and T.P. Kellner. 1983. Primary Dermal Irritation Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge. Institute Report No. 159, AD A133084. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The Holston compounds designated DMSO recycle solvent (TP013), virgin DMSO (TP014), and DMSO evaporator sludge (TP015) were tested for primary dermal irritation potential on rabbits. The study was conducted in compliance with the Good Laboratory Practice Regulations. While all three test compounds caused slight erythema on a few animals, the average scores were low enough for all three compounds to be classified as nonirritating after one application.

8. C.M. Lewis, Y.C. Johnson, and D.W. Korte, Jr. 1984. Dermal Sensitization Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge. Institute Report No. 172, AD A142926. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The Holston Compounds designated virgin DMSO (TP014), DMSO recycle solvent (TP013), and DMSO evaporator sludge (TP015) were tested for dermal sensitization potential on guinea pigs. The study was conducted in compliance with the Good Laboratory Practice Regulations. The results from this study indicate that the test compounds are mild irritants (under conditions of the study) and provide little evidence of a sensitizing potential.

9. Johnson, Y.C., C.M. Lewis, and D.W. Korte, Jr. 1984. Dermal Sensitization of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine. Institute Report No. 183, AD A146826. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The explosive by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), was tested for dermal sensitization potential on guinea pigs. The study was conducted in compliance with the Good Laboratory Practice Regulations. The absence of erythema in the test animals during the study indicated SEX is a nonsensitizer when applied topically in saline according to the closed patch dermal sensitization technique of Buehler.

10. Sauers, L.J., T.P. Kellner, J.T. Fruin. 1983. Mutagenic Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, DMSO Evaporator Sludge. Institute Report No. 149, AD A130159. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The mutagenic potential of the Holston compounds (virgin DMSO, DMSO recycle solvent, and DMSO evaporator sludge) was assessed by using the Ames Salmonella/mammalian microsome mutagenicity assay. Tester strains TA98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 0.1 mL of a 100 percent to 0.1 mL of a 0.032 percent solution. Negative mutagenic responses were observed for the virgin DMSO and the DMSO recycle solvent. Mutagenic potential was observed for the DMSO evaporator sludge.

11. Kellner, T.P., L.J. Sauers, and J.T. Fruin. 1983. Mutagenic Potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) and 1-acetylhexahydro-3,5-dinitro-1,3,5-triazine (TAX). Institute Report No. 165, AD A137109. Letterman Army Institute of Research, Presidio of San Francisco, CA.

The mutagenic potential of SEX and TAX was assessed by using the Ames Salmonella/mammalian microsome mutagenicity assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 mg/plate to 10^{-5} mg/plate. Negative mutagenic responses were observed for both test compounds.

12. Sano, S.K. and D.W. Korte, Jr. 1984. Mutagenic Potential of Benzothiazole (TA037). Letterman Army Institute of Research, Presidio of San Francisco, CA. DRAFT

The mutagenic potential of benzothiazole was assessed by using the Ames Salmonella/mammalian microsome mutagenicity assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1 μ L/plate to a 3×10^{-4} μ L/plate. The test compound was not mutagenic under conditions of this assay.

13. Kawakami, T.G. and A. Aotaki-Keen. 1983. Mutagenic Activity of Dimethylsulfoxide (DMSO) Solvent Samples from Munition Pilot Test Plant on Mammalian Cells. Final Report, AD A141024. Project No. APO-3801. Laboratory for Energy-Related Health Research, University of California, Davis, CA.

Two DMSO solvent samples containing munition constituents contained a direct-acting mutagen(s). Of the two samples, DMSO recycle solvent sample had a more acute mutagenic activity than DMSO evaporator sludge sample at comparable dilution of the stock solution. The mutagenic activity in these samples was not augmented with addition of rat liver S-9. Since these samples are a mixture of several compounds, the identity of the specific compound(s) responsible for the mutagenic activity is not known.

14. Burrows, E.P. and E.E. Brueggemann. 1984. Chemical Characterization of Dimethylsulfoxide (DMSO) Munitions Recrystallization Process Samples. Technical Report 8407, AD A149103. US Army Medical Bioengineering Research & Development Laboratory, Fort Detrick, Frederick, MD.

As part of a program to assess possible adverse health effects to workers in a new nitramine munitions recrystallization process utilizing dimethyl sulfoxide, pilot plant samples were analyzed qualitatively for trace organics by gas chromatography/mass spectrometry and quantitatively for nitramines by high pressure liquid chromatography. None of the variety of trace organics found was related to munitions manufacture or to breakdown of nitramines, and, except for the presence of diacetone alcohol, there was little overall consistency in trace organic content of the samples.

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1	Library Technician US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBZ-IL Fort Detrick, Frederick, MD 21701-5010
2	Commander US Army Materiel Command ATTN: AMCSG 5001 Eisenhower Ave. Alexandria, VA 22333-0001
2	Commander US Army Armament, Munitions and Chemical Command ATTN: AMSMC-SG Rock Island, IL 61299-5000
2	Commander US Army Armament, Munitions and Chemical Command ATTN: AMSMC-LCM-E(D) Dover, NJ 07801
2	Commander US Army Armament, Munitions and Chemical Command ATTN: AMSPM-PBM-EG(D) Rock Island, IL 61299-5000
2	Commander US Army Environmental Hygiene Agency ATTN: HSHB-OM Aberdeen Proving Ground, MD 21010-5422

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